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25(OH)D₃-enriched or fortified foods are more efficient at tackling inadequate vitamin D status than vitamin D₃

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The ability to synthesise sufficient vitamin D through sunlight in human subjects can be limited. Thus, diet has become an important contributor to vitamin D intake and status; however, there are only a few foods (e.g. egg yolk, oily fish) naturally rich in vitamin D. Therefore, vitamin D-enriched foods via supplementing the animals’ diet with vitamin D or vitamin D fortification of foods have been proposed as strategies to increase vitamin D intake. Evidence that cholecalciferol (vitamin D₃) and calcifediol (25(OH)D₃) content of eggs, fish and milk increased in response to vitamin D₃ supplementation of hens, fish or cows’ diets was identified when vitamin D-enrichment studies were reviewed. However, evidence from supplementation studies with hens showed only dietary 25(OH)D₃, not vitamin D₃ supplementation, resulted in a pronounced increase of 25(OH)D₃ in the eggs. Furthermore, evidence from randomised controlled trials indicated that a 25(OH)D₃ oral supplement could be absorbed faster and more efficiently raise serum 25(OH)D concentration compared with vitamin D₃ supplementation. Moreover, evidence showed the relative effectiveness of increasing vitamin D status using 25(OH)D₃ varied between 3.13 and 7.14 times that of vitamin D₃, probably due to the different characteristics of the investigated subjects or study design. Therefore, vitamin D-enrichment or fortified foods using 25(OH)D₃ would appear to have advantages over vitamin D₃. Further well-controlled studies are needed to assess the effects of 25(OH)D₃ enriched or fortified foods in the general population and clinical patients.

Enrichment: Fortification: 25(OH)D₃: Vitamin D₃: Vitamin D deficiency

Vitamin D is usually synthesised in skin that is exposed to UV radiation, which has led to the term ‘sunshine vitamin’⁽¹⁾. Traditionally, the primary role of vitamin D is related to calcium absorption and bone health. Children and adults with vitamin D deficiency have an increased risk of developing rickets or osteomalacia⁽²⁾. Recently, a resurgence of childhood rickets has highlighted the need for adequate vitamin D status in many parts of the

world^(3–5). Furthermore, mounting evidence from epidemiological studies indicates that vitamin D status is inversely associated with the risk of CVD, cancers and diabetes^(1,6), although there is some uncertainty about what defines an adequate vitamin D status⁽⁷⁾.

Vitamin D deficiency is prevalent and is considered a serious issue throughout the world^(8–10), even in sunnier climates such as Australia and New Zealand⁽¹¹⁾.

Abbreviations: 25(OH)D₃, 25-hydroxyvitamin D₃; RCT, randomised controlled trial.

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Recently, the Scientific Advisory Committee on Nutrition⁽⁷⁾ reported that in the UK, 22–24 % of adults aged 19–64 years, and 17–24 % of those ≥ 65 years were vitamin D deficient (plasma 25-hydroxyvitamin D₃ (25(OH)D₃) <25 nmol/l). There are several factors that have contributed to the low vitamin D status commonly seen today, such as lifestyle changes (increased indoor lifestyle, sun screens use) and human characteristics (e.g. ageing, clothing, increased obesity, low-fat diet trend)⁽¹²⁾. Therefore, foods that contribute to vitamin D intake have become more important than before. However, there are only a few foods naturally rich in vitamin D, such as oily fish and egg yolks⁽¹³⁾.

The aim of this review is first to critically evaluate the existing evidence on whether the vitamin D content of animal-derived foods can be increased by feeding cholecalciferol (vitamin D₃) and/or calcifediol (25(OH)D₃) supplements to laying hens, fish and cows. Second, the present review summarises evidence from the human randomised controlled trials (RCT), which include the effects of 25(OH)D₃ supplementation on increasing serum/plasma 25(OH)D₃ concentration.

Vitamin D absorption, synthesis and metabolism

Generally, the term vitamin D refers to both vitamin D₂ and vitamin D₃. Vitamin D₂ is produced by fungi, while vitamin D₃ is produced by human subjects and animals⁽¹⁴⁾. Human subjects usually synthesise vitamin D₃ in the skin⁽¹⁵⁾ where 7-dehydrocholesterol in the epidermis is converted to pre-vitamin D₃ when skin is exposed to sunlight. Then, pre-vitamin D₃ undergoes a temperature-dependent isomerisation to vitamin D₃ over a period of approximately 3 d⁽⁶⁾. Vitamin D (vitamin D₂ or vitamin D₃) can also be obtained from the diet⁽¹⁵⁾ and it is absorbed with long-chain TAG in the small intestine⁽¹⁶⁾. It is then incorporated into chylomicrons and transported in lymph to the blood and into the general circulation⁽¹⁷⁾.

After entering the circulation, there are two hydroxylation reactions to convert vitamin D to the biologically active form⁽⁶⁾. The first hydroxylation reaction is in the liver where vitamin D is hydroxylated to 25(OH)D by the vitamin D-25-hydroxylase enzyme. The second hydroxylation reaction is in the kidney where 25(OH)D is converted to 1,25(OH)₂D by 25-hydroxyvitamin D-1 α -hydroxylase⁽⁶⁾, and the 1,25(OH)₂D metabolite is the biologically active form of vitamin D⁽¹⁸⁾.

Foods of animal origin as dietary sources of vitamin D

Vitamin D content of vitamin D-enriched foods can differ considerably between food retailers. One US retail study analysed the vitamin D content of egg yolks collected from twelve individual retail supermarkets across the country and reported a broad range of vitamin D₃ and 25(OH)D₃ concentrations of 9.7–18 and 4.3–13.2 $\mu\text{g/kg}$, respectively⁽¹⁹⁾. In addition, our recent UK retail study⁽²⁰⁾ showed vitamin D₃ and 25(OH)D₃

concentrations of eggs were significantly different depending on the egg production systems. Egg yolks produced by birds kept in indoor systems had much lower concentrations (40.2 (SE 3.1) $\mu\text{g/kg}$) of vitamin D₃ than the egg yolks produced from outdoor systems (57.2 (SE 3.2) $\mu\text{g/kg}$), while 25(OH)D₃ concentrations of the eggs were higher in organic eggs only. Similarly, the vitamin D contents of fish have been shown to vary according to the production systems. The study of Lu *et al.*⁽²¹⁾ indicated the vitamin D₃ content of wild salmon to be three times higher than that of farmed salmon; however, the 25(OH)D₃ content of the salmon was not measured. In addition, other studies^(22,23) have shown the 25(OH)D₃ content of several species of marine and freshwater fish to be <0.02 $\mu\text{g/100 g}$. Therefore, foods generally regarded as rich sources of vitamin D may not be sustainable vitamin D contributors for the general population, due to variability in vitamin D content, which in turn may be influenced by production systems or different species (genotype). Furthermore, the National Diet and Nutrition Survey of the UK⁽²⁴⁾ reported that the average daily intake of vitamin D for adults was 3.1 μg for men and 2.6 μg for women, which is much lower than the UK vitamin D reference nutrition intake of 10 $\mu\text{g/d}$ ⁽⁷⁾. Therefore, vitamin D-enriched or fortified foods are needed to ensure an adequate vitamin D intake for the general population.

Enrichment of animal-derived foods as dietary sources of vitamin D

Vitamin D-enriched eggs

In general, there are two main methods to enrich the vitamin D content of eggs: increased sunlight exposure and vitamin D supplementation of the birds' diet. Because hens can synthesise vitamin D from natural sunlight exposure, free-range egg production system may be an inexpensive way to increase their vitamin D content. A study by Kuhn *et al.* assigned laying hens to a free-range treatment or an indoor treatment for over 4 weeks and found that eggs from the free-range group, which were exposed to sunlight, had significantly higher vitamin D₃ content (mean 14.3 $\mu\text{g/100 g DM}$) than eggs from the indoor group (mean 3.8 $\mu\text{g/100 g DM}$)⁽²⁵⁾. Furthermore, there are several studies which have shown that the vitamin D₃ content of eggs can be enhanced by feeding vitamin D₃ supplements to the hens (Table 1)^(26–32). The results of all studies revealed that egg yolk vitamin D₃ concentration was efficiently increased by vitamin D₃ dietary supplementation. The study of Yao *et al.* showed a linear dose–response relationship existed between vitamin D₃ dietary supplementation and vitamin D₃ concentrations of egg yolks⁽³⁰⁾. Moreover, as 25(OH)D₃ is a metabolite of vitamin D₃, the 25(OH)D₃ content in eggs can also be enhanced by supplementing the birds' diet with vitamin D₃. However, the response in 25(OH)D₃ content of egg yolk is much less than that of vitamin D₃. Browning and Cowieson⁽³¹⁾ showed that a 4-fold increase in vitamin D₃, and a 2-fold increase in 25(OH)D₃ in egg yolk resulted from a 4-fold increase in the vitamin D₃

Table 1. Summary of enrichment studies investigating the impact of adding vitamin D to the diet of laying hens on the vitamin D content of egg yolks

References	Vitamin D supplement (µg/kg)		Feeding duration (weeks)	Vitamin D concentration of egg yolk (µg/100 g)	
	Vitamin D ₃	25(OH)D ₃		Vitamin D ₃	25(OH)D ₃
Mattila <i>et al.</i> ⁽²⁶⁾	26.6	–	6	1.4	0.5
	62.4	–	6	3.5	0.9
	216.0	–	6	22.0	1.5
Mattila <i>et al.</i> ⁽²⁷⁾	280.0	–	4	30.0	1.9
Mattila <i>et al.</i> ⁽²⁸⁾	62.5	–	4	3.8	–
	150.0	–	4	13.6	–
	375.0	–	4	33.7	–
Browning and Cowieson ⁽³¹⁾	62.5	–	9	6.5	1.6
	125.0	–	9	10.5	2.1
	250.0	–	9	26.2	3.0
Yao <i>et al.</i> ⁽³⁰⁾	55.0	–	3	3.0	–
	242.5	–	3	21.6	–
	430.0	–	3	41.0	–
	617.5	–	3	60.3	–
	2555.0	–	3	870.4	–
Browning and Cowieson ⁽³¹⁾	62.5	0	9	6.5	1.6
	62.5	34.5	9	6.0	3.3
	62.5	69.0	9	4.9	4.5
	125.0	0	9	10.5	2.1
	125.0	34.5	9	7.4	4.5
	125.0	69.0	9	8.1	5.8
	250.0	0	9	26.2	3.0
	250.0	34.5	9	23.6	3.7
	250.0	69.0	9	30.9	8.1
Mattila <i>et al.</i> ⁽²⁹⁾	–	55.0	6	≤0.2	2.1
	–	122.0	6	≤0.2	4.3
Duffy <i>et al.</i> ⁽³²⁾	37.5	–	4	1.0*	1.9*
	75.0	–	4	2.0*	1.9*
	37.5	37.5	4	1.3*	3.6*
		75.0	4	0.7*	4.4*

25(OH)D₃, 25-hydroxyvitamin D₃.

* Vitamin D content per egg.

in the diet (62.5–250 µg/kg). Similarly, evidence from another study showed that the vitamin D₃ in egg yolk was increased approximately 7-fold as a result of feeding a diet with a 3.5-fold higher vitamin D₃ content (from 62.4 to 216 µg/kg), while the corresponding increase in 25(OH)D₃ content was only about 1.5-fold⁽²⁶⁾.

There are only a few studies^(29,31,32) examining the effect of feeding birds with diets supplemented with 25(OH)D₃. In the EU, 25(OH)D₃ has only recently been authorised for addition to poultry diets, and the maximum content of the vitamin D₃ and 25(OH)D₃ combination for laying hens is 80 µg/kg^(33,34). It is of note that most of vitamin D supplementation studies^(27–31), summarised in Table 1, had higher vitamin D doses than the EU diet limit⁽³³⁾, thus, the potential for increasing vitamin D in eggs by adding vitamin D to the diet of laying hens is limited by EU regulations. Browning and Cowieson⁽³¹⁾ and Duffy *et al.*⁽³²⁾ both showed an addition of 25(OH)D₃ to the vitamin D₃ supplement resulted in the elevation of the 25(OH)D₃ content of the egg yolk, but there was no significant increase in the vitamin D₃ content of the egg yolk. Other studies investigated dietary supplementation with 25(OH)D₃^(29,32), and showed that

only egg yolk 25(OH)D₃ was increased, but not vitamin D₃. Therefore, we speculate that 25(OH)D₃ in the diet can be absorbed directly by laying hens without transfer to vitamin D₃ in the circulation.

Vitamin D-enriched fish

There are very few studies on enriching the vitamin D content of fish (Table 2)^(35–38). Mattila *et al.* fed rainbow trout with different doses of vitamin D₃ supplements up to 539 µg/kg, but no significant differences in the vitamin D₃ content of the fish fillet were observed⁽³⁷⁾. In contrast, the study of Horvli *et al.* with Atlantic salmon showed a dose–response relationship between the vitamin D₃ in the diet up to 28.68 mg/kg and vitamin D₃ in the fish meat⁽³⁵⁾. Similar high vitamin D₃ supplementation doses were reported in another two studies^(36,38), which also showed that elevated vitamin D₃ content of the fish liver or whole fish had been achieved by supplemental vitamin D₃ in the diet. However, 25(OH)D₃ contents of the enriched fish were not measured in these studies^(35–38), and the lack of evidence on the effects by feeding fish with 25(OH)D₃ on the vitamin D content of the

Table 2. Summary of enrichment studies investigating the impact of vitamin D supplemental fish feeding on vitamin D content of fish

References	Vitamin D ₃ supplement (µg/kg)	Feeding duration (weeks)	Vitamin D ₃ of fish (µg/100 g)
Horvli <i>et al.</i> ⁽³⁵⁾	40	11	1 (fillet)
	2210	11	21 (fillet)
	28 680	11	210 (fillet)
Vielma <i>et al.</i> ⁽³⁶⁾	62.5	12	1 (liver)
	6250	12	73 (liver)
	62 500	12	6900 (liver)
Mattila <i>et al.</i> ⁽³⁷⁾	89	16	6–15 (fish fillet)
	174	16	6–10 (fish fillet)
	539	16	7–16 (fish fillet)
Graff <i>et al.</i> ⁽³⁸⁾	200	9	≤25 (whole fish)*
	5000	9	80 (whole fish)*
	57 000	9	650 (whole fish)*

* Estimated from graph.

fish warrants further research. Again, supplement doses of the listed studies^(35–38) in Table 2 were over the EU diet limit for farmed fish of 75 µg/kg⁽³³⁾, which will limit application in the market.

Vitamin D-enriched milk

A few studies have investigated the longer term effect of supplemental vitamin D₃ on the vitamin D content of the milk; the summary of these studies is presented in Table 3^(39–42). Hollis *et al.* showed a 10-fold enhancement of vitamin D₃ intake from 100 to 1000 µg/d resulted in a 7.5-fold increased vitamin D₃ concentration of the milk and a 2-fold increase in 25(OH)D₃⁽³⁹⁾. Moreover, McDermott *et al.* compared three different doses of vitamin D₃ with a control diet, and showed an increased concentration of vitamin D₃ and 25(OH)D₃ in the milk⁽⁴¹⁾. However, the relationship between increasing dietary vitamin D₃ doses and milk vitamin D₃ or 25(OH)D₃ concentrations were not linear. Furthermore, the study of Weiss *et al.* investigated the effect of feeding 450 µg/d vitamin D₃ to pre-calving cows for 13 d which resulted in concentrations of vitamin D₃ and 25(OH)D₃ in the milk ranging from 0.33–0.45 to 0.36–1.02 µg/l, respectively⁽⁴²⁾. In addition, the study included a diet treatment of 6 mg vitamin D₃ with a cation–anion difference of –138 mEq/kg daily for 13 d; the concentrations of 25(OH)D₃ in the milk were increased but the treatment effect disappeared after 28 d. Therefore, evidence from the limited number of studies^(39–42) demonstrated that milk vitamin D concentrations can be increased by feeding dairy cows with vitamin D supplements. However, it is of note that the highest milk vitamin D₃ and 25(OH)D₃ concentrations were 0.47 and 3.69 µg/l, respectively (Table 3), which for one typical milk serving of 200 ml only contributes 0.09 and 0.74 µg vitamin D₃ and 25(OH)D₃, respectively, well below the current UK vitamin D reference nutrition intake of 10 µg/d⁽⁷⁾. Furthermore, the doses of vitamin D in those studies^(41,42) were much higher than the maximum allowed vitamin D content

in EU (0.01 mg/kg diet at 880 g DM/kg approximately equivalent to 2.27 mg/d)⁽³⁴⁾, which imposes an even greater restriction on the possibility of increasing vitamin D in milk by adding vitamin D supplements in the diet of dairy cows.

Evidence from human dietary intervention studies with vitamin D-enriched animal-derived foods

Despite numerous animal-based vitamin D-enrichment studies on vitamin D in eggs, fish and milk, there are few RCT on the effect of consuming vitamin D-enriched foods on the vitamin D status of the consumer. To our knowledge, only one recent study has investigated the weekly effect of consuming seven vitamin D₃ or seven 25(OH)D₃-enriched eggs on vitamin D status compared with commercial eggs of ≤2 egg/week⁽⁴³⁾. After 8 weeks follow-up in winter, the results showed that while the serum 25(OH)D of the subjects who consumed commercial eggs decreased from a baseline of 41 (SD 14.1) nmol/l to 35 (SD 11.4) nmol/l, the serum 25(OH)D of subjects who consumed vitamin D₃-enriched eggs or 25(OH)D₃-enriched eggs was maintained. The serum 25(OH)D concentrations of subjects who consumed vitamin D₃- or 25(OH)D₃-enriched eggs were 50 (SD 21.4) nmol/l and 49 (SD 16.5) nmol/l, respectively. However, there was no significant difference between vitamin D₃- and 25(OH)D₃-enriched egg consumption on serum 25(OH)D concentrations.

Although there are a limited number of human dietary intervention studies on vitamin D-enriched foods, the study of Mattila *et al.*⁽²⁹⁾ demonstrated that the effect of foods enriched with either vitamin D₃ or 25(OH)D₃ on human vitamin D status depended on their relative effectiveness of raising serum or plasma 25(OH)D concentrations. A previous study⁽⁴⁴⁾ indicated that there was no consensus on the relative effectiveness of 25(OH)D₃ compared with vitamin D₃ for raising human serum or plasma 25(OH)D₃ concentrations. Furthermore, UK food composition tables⁽⁴⁵⁾ indicate that there is no certainty on the relative potency of 25(OH)D₃ compared with vitamin D₃, although it was assumed that 25(OH)D₃ had a potency of five times that of vitamin D₃ for calculating the total vitamin D of foods⁽⁴⁵⁾.

Human intervention studies on the relative effects of calcifediol and cholecalciferol supplementation on vitamin D status

Heterogeneity of intervention studies

Eleven RCT that investigated the effects of 25(OH)D₃ relative to vitamin D₃ were identified^(46–56) (Table 4). Nine studies administered 25(OH)D₃ supplementation only, except two studies which provided a combination supplement of 25(OH)D₃ and calcium^(46,49). Five of the eleven studies^(47,49–52) supplemented 25(OH)D₃ to generally healthy subjects, whereas the other six studies^(46,48,53–56) supplemented 25(OH)D₃ to clinical patients. Most studies reported the serum or plasma

Table 3. Summary of enrichment studies investigating the impact of vitamin D supplementation to the diet of dairy cows on vitamin D content of milk

References	Supplements to diet (µg/d)			Vitamin D concentration of milk (µg/l)		
	Vitamin D ₃	25(OH)D ₃	Feeding duration	Vitamin D ₃	25(OH)D ₃	1,25(OH) ₂ D ₃
Hollis <i>et al.</i> ⁽³⁹⁾	100	–	NA	0.04	0.37	0.01
	1000	–	NA	0.32	0.68	0.004
Reeve <i>et al.</i> ⁽⁴⁰⁾	375	–	30 d	0.28	0.15	0.01
Mcdermott <i>et al.</i> ⁽⁴¹⁾	0	–	14 weeks	0.08	0.25	0.10
	250	–	14 weeks	0.20	0.43	0.03
	1250	–	14 weeks	0.15	0.75	0.13
	6250	–	14 weeks	0.33	0.93	0.10
Weiss <i>et al.</i> ⁽⁴²⁾	450	–	13 d before calving	0.33–0.47	0.36–1.02	–
	–	DCAD + 6000	13 d before calving	–	0.61–3.69	–

25(OH)D₃, 25-hydroxyvitamin D₃; 1,25(OH)₂D₃, 1,25 dihydroxyvitamin D₃; DCAD, dietary cation–anion difference of –138 mEq/kg.

25(OH)D concentration at both the beginning and end of the treatment, except one study⁽⁵⁵⁾, which only reported the 25(OH)D concentration at the end of the treatment. In terms of the vitamin D status measurement, most studies measured total 25(OH)D concentration, except two studies^(49,52), which measured 25(OH)D₃. For the characteristics of the investigated subjects, five studies included both men and women^(46,48,51,53,55), while the other studies only included men or women. In addition, most studies reported the age and BMI of the subjects, except two studies^(46,48) that did not report the BMI range.

Acute pharmacokinetic action of cholecalciferol and calcifediol

An early study provided meals with single doses of 25(OH)D₃ of 1.5, 5 or 10 µg/kg body weight to generally healthy subjects and showed that the peak serum 25(OH)D₃ concentration was reached within 4–8 h after ingestion⁽⁵⁷⁾. A later study by Jetter *et al.* compared the pharmacokinetic absorption of vitamin D₃ and 25(OH)D₃ by providing a single dose of 20 µg vitamin D₃ or 20 µg 25(OH)D₃ to postmenopausal women⁽⁵²⁾. The time to reach maximum plasma 25(OH)D₃ concentration was 22 and 11 h for vitamin D₃ and 25(OH)D₃, respectively. In addition, the peak concentration of plasma 25(OH)D₃ (44 nmol/l) from 25(OH)D₃ supplementation was higher than vitamin D₃ supplementation (35 nmol/l), although they were not significantly different. This study further compared the effect of a higher single dose of 140 µg vitamin D₃ and 140 µg 25(OH)D₃ with the time to reach peak plasma 25(OH)D₃ being 21 and 4–8 h for vitamin D₃ and 25(OH)D₃ supplementation, respectively⁽⁵²⁾. In addition, the maximum plasma concentration of 25(OH)D₃ for 25(OH)D₃ treatment (100 nmol/l) was significantly higher than for vitamin D₃ treatment (44 nmol/l). These results suggest that 25(OH)D₃ was absorbed more quickly than vitamin D₃ possibly because 25(OH)D₃ has higher solubility in aqueous media than vitamin D₃ due to its more polar chemical structure⁽⁵⁸⁾. Furthermore, as this metabolite of vitamin D₃ is produced in the liver, the hepatic metabolism of vitamin D₃ to 25(OH)D₃ is circumvented and consequently the

conversion from vitamin D₃ to 25(OH)D₃ would be negligible⁽⁵⁹⁾. In patients with liver disease who had an impaired ability to synthesise 25(OH)D₃ from vitamin D₃⁽⁶⁰⁾, the study of Sitrin and Bengoa⁽⁶¹⁾ verified that 25(OH)D₃ could be absorbed more efficiently than vitamin D₃ after oral supplementation. Therefore, supplementation with 25(OH)D₃ is not only more efficient at increasing vitamin D status in generally healthy people, but may also have a specific role in tackling lower vitamin D status in patients who are suffering from liver diseases.

Chronic effects and relative effectiveness of cholecalciferol and calcifediol treatments

Regarding the expected higher biological effect of 25(OH)D₃ in raising serum or plasma 25(OH)D level after long-term administration, several studies have confirmed that oral consumption of 25(OH)D₃ is highly effective in raising serum or plasma 25(OH)D level (Table 4)^(46–56). However, the majority of the evidence in support of a higher impact of 25(OH)D₃ supplementation compared with vitamin D₃ on serum or plasma 25(OH)D₃ level is from only four studies^(51,52,54,56) where both 25(OH)D₃ and vitamin D₃ treatments were included in the same study (Table 5). The study of Barger-Lux *et al.*⁽⁴⁷⁾ provided three different doses of vitamin D₃ (25, 250, 1250 µg/d) or 25(OH)D₃ (10, 20, 50 µg/d) to the participants for 8 and 4 weeks, respectively. However, the effects of 25(OH)D₃ and vitamin D₃ treatments were not directly comparable as the interventions were not at the same dose or treatment time. Thus, the study of Barger-Lux *et al.*⁽⁴⁷⁾ was excluded from the relative effectiveness analysis. In order to compare the relative effectiveness of 25(OH)D₃ and vitamin D₃ supplementation on raising serum or plasma 25(OH)D concentrations, a dose–response factor was calculated for each µg of orally consumed 25(OH)D₃ or vitamin D₃ in four studies^(51,52,54,56). The dose–response factors of 25(OH)D₃ and vitamin D₃ were calculated by using endpoint 25(OH)D concentration minus baseline 25(OH)D concentration, divided by the dose of the supplementation (dose–response factor = Δ serum/plasma (mmol/l)/dose (µg)). Then, the relative



Table 4. Summary of study details and serum 25, hydroxyvitamin D (25(OH)D) concentration in long-term randomised controlled trials with calcifediol (25 hydroxyvitamin D₃ (25(OH)D₃)) supplementation in adults (order by year)

References	Subjects characteristics (trial time during the year, subjects (sex), age, BMI)	25(OH)D ₃ supplementation group					Control group (if available)				
		Duration	25(OH)D ₃ treatment	n	Baseline 25(OH)D (nmol/l)	Endpoint 25(OH)D (nmol/l)	Duration	Vitamin D ₃ treatment	n	Baseline 25(OH)D (nmol/l)	Endpoint 25(OH)D (nmol/l)
Hahn <i>et al.</i> ⁽⁴⁶⁾	Whole year, patients (women and men) with glucocorticoid-induced osteopenia 46 years, BMI (NA*)	18 months	40 µg/d + 500 mg calcium/d	9	39	205					
Barger-Lux <i>et al.</i> ⁽⁴⁷⁾	January–April, men 28 years, 26 kg/m ²	4 weeks	10 µg/d	7	67	107	8 weeks	25 µg/d	13	67	96
		4 weeks	20 µg/d	6	67	143	8 weeks	250 µg/d	10	67	213
		4 weeks	50 µg/d	4	67	273	8 weeks	1250 µg/d	14	67	710
Jean <i>et al.</i> ⁽⁴⁸⁾	March–September, haemodialysis patients (women and men) 67 years, BMI (NA)	6 months	16 µg/d	149	30	126					
Cavalli <i>et al.</i> ^{§(49)}	April–July, postmenopausal women 65–75 years, 25 kg/m ²	12 weeks	125 µg/week + 500 mg calcium/d	25	50	76					
		12 weeks	250 µg/month + 500 mg calcium/d	28	51	70					
		12 weeks	500 µg/month + 500 mg calcium/d	27	52	77					
Russo <i>et al.</i> ⁽⁵⁰⁾	January–April, women (7 premenopausal and 11 postmenopausal), 24–72 years, 24 kg/m ²	16 weeks	500 µg/month	18	45	105 [†]					
Cashman <i>et al.</i> ⁽⁵¹⁾	January–April, women and men, 57 years, 29 kg/m ²	10 weeks	20 µg/d	12	38	135	10 weeks	20 µg/d	13	50	69
Jetter <i>et al.</i> ^{‡§(52)}	January–July, postmenopausal women 50–70 years, 18–29 kg/m ²	16 weeks	20 µg/d	5	31	173	16 weeks	20 µg/d	5	35	77
Catalano <i>et al.</i> ⁽⁵⁴⁾	September–March, osteopenic and dyslipidaemic postmenopausal women 59 years, 27 kg/m ²	24 weeks	140 µg once weekly	29	56	126	24 weeks	140 µg once weekly	28	51	61
Banon <i>et al.</i> ⁽⁵³⁾	Whole year, patients (women and men) had HIV-infected, 44 years, 15–44 kg/m ²	Summer	400 µg once/month	123	37	86	Summer	NA	242	53	99
		Fall	400 µg once/month	123	37	69	Fall	NA	242	53	84
		Winter	400 µg once/month	123	37	45	Winter	NA	242	53	55
		Spring	400 µg once/month	123	37	57	Spring	NA	242	53	78
Ortego-Jurado <i>et al.</i> ⁽⁵⁵⁾	Whole year, patients (women and men) had autoimmune diseases, undergoing glucocorticoids therapy, 56 years, 28 kg/m ²	Spring–summer	8.85 µg/d	49	NA	84	Spring–summer	20 µg/d	86	NA	71
		Fall–winter	8.85 µg/d	49	NA	89	Fall–winter	20 µg/d	86	NA	61
Navarro-Valverde <i>et al.</i> ⁽⁵⁶⁾	Whole year, postmenopausal osteoporotic women, 67 years, 26 kg/m ²	6 months	20 µg/d	10	37	161	6 months	20 µg/d	10	41	80
		12 months	20 µg/d	10	37	188	12 months	20 µg/d	10	41	86
		6 months	266 µg once/week	10	38	214					
		12 months	266 µg once/week	10	38	233					
		6 months	266 µg once/2 weeks	10	40	165					
		12 months	266 µg once/2 weeks	10	40	211					

* NA, not available.

† Estimated from graph.

‡ Same study of (Jetter *et al.*⁽⁵²⁾) and (Bischoff-Ferrari *et al.*⁽⁶²⁾).

§ Study has measured vitamin D status as 25(OH)D₃.

Table 5. Summary of randomised controlled trials with both calcifediol (25 hydroxyvitamin D₃ (25(OH)D₃)) and vitamin D₃ in adults to calculate the relative effectiveness of 25(OH)D₃ and vitamin D₃ supplementation in raising serum 25, hydroxyvitamin D (25(OH)D) level

References	Treatment (dose, duration)	Serum 25(OH)D raising (nmol/l) per 1 µg*	Relative effectiveness†
Cashman <i>et al.</i> ⁽⁵¹⁾	20 µg 25(OH)D ₃ /d × 10 weeks	4.82 ^a	4.99
	20 µg vitamin D ₃ /d × 10 weeks	0.97 ^b	
Jetter <i>et al.</i> ⁽⁵²⁾	20 µg 25(OH)D ₃ /d × 15 weeks	7.12 ^a	3.40
	20 µg vitamin D ₃ /d × 15 weeks	2.51 ^b	
Catalano <i>et al.</i> ⁽⁵⁴⁾	140 µg 25(OH)D ₃ /week × 24 weeks	0.50 ^a	7.14
	140 µg vitamin D ₃ /week × 24 weeks	0.07 ^b	
Navarro-Valverde <i>et al.</i> ⁽⁵⁶⁾	20 µg 25(OH)D ₃ /d × 6 months	6.19 ^a	3.13
	20 µg vitamin D ₃ /d × 6 months	1.98 ^b	
	20 µg 25(OH)D ₃ /d × 12 months	7.54 ^a	
	20 µg vitamin D ₃ /d × 12 months	2.29 ^b	

* Dose–response factor = Δ serum/plasma (nmol/l)/dose (µg).

† Relative effectiveness = a/b within same study.

effectiveness of 25(OH)D₃ to vitamin D₃ was calculated by dividing the dose–response factor of 25(OH)D₃ by that of vitamin D₃.

The highest relative effectiveness was found in the study by Catalano *et al.*⁽⁵⁴⁾. Weekly treatment of 140 µg 25(OH)D₃ or 140 µg vitamin D₃ supplements was provided to osteopenic and dyslipidaemic postmenopausal women for 24 weeks. Supplementation with 25(OH)D₃ raised serum 25(OH)D from a baseline of 56–126 nmol/l, while vitamin D₃ treatment increased serum 25(OH)D to a lower extent, from baseline 51 to 61 nmol/l. Thus, the relative effectiveness factor derived from this study was 7.14, i.e. dietary 25(OH)D₃ was 7.14 times more effective at increasing serum 25(OH)D than dietary vitamin D₃.

Vitamin D dietary recommendations are generally between 10 and 20 µg/d⁽¹⁰⁾, yet, there are few studies which have compared the effectiveness of dietary 25(OH)D₃ and vitamin D₃ using doses of 20 µg in their treatments. Cashman *et al.*⁽⁵¹⁾ provided daily supplements of 20 µg vitamin D₃ or 20 µg 25(OH)D₃ to adult men and women with a mean age of 57 years and with baseline serum 25(OH)D of 28.9 nmol/l during winter. After 10 weeks of supplementation, the subjects' serum 25(OH)D increased to 135 and 69 nmol/l for the 25(OH)D₃ and vitamin D₃ treatments, respectively. A relative effectiveness factor of 4.99 was calculated representing the relative effectiveness of each µg of dietary 25(OH)D₃ relative to dietary vitamin D₃ for raising serum 25(OH)D concentration. However, lower relative effectiveness factors were achieved in other studies using the same dose of 20 µg vitamin D₃ and 25(OH)D₃. Jetter *et al.* supplemented healthy postmenopausal women with 20 µg 25(OH)D₃ or 20 µg vitamin D₃ for 16 weeks during the winter⁽⁵²⁾. They found that for the 25(OH)D₃ treatment, plasma 25(OH)D₃ increased to 173 nmol/l from a baseline of 31 nmol/l, whereas for the vitamin D₃ treatment, plasma 25(OH)D₃ increased to 77 nmol/l from a baseline level of 35 nmol/l. The relative effectiveness factor of each µg of 25(OH)D₃ was 3.40 compared with vitamin D₃ in raising plasma 25(OH)D₃ level. A similar low relative effectiveness factor was found in another study where post-menopausal osteoporotic women were given either 20 µg vitamin D₃ or 20 µg

25(OH)D₃ over 6 or 12 months⁽⁵⁶⁾. The serum concentration of 25(OH)D for the 25(OH)D₃ treatment reached 161 and 188 nmol/l from a baseline of 37 nmol/l after 6 or 12 months administration, respectively, while the comparable values for the vitamin D₃ treatment were an increase to 80 and 86 nmol/l from a baseline of 41 nmol/l. So the relative effectiveness factor of 25(OH)D₃ relative to vitamin D₃ treatment at 6 and 12 months were 3.13 or 3.29, respectively.

In summary, of the studies reviewed, the relative effectiveness of 25(OH)D₃ to vitamin D₃ for raising vitamin D status (Table 5), ranged from 3.13 to 7.14. Previous studies have demonstrated that the season may have influences on vitamin D status^(13,14). There were two studies conducted during the winter which may have minimised any confounding influence of cutaneous vitamin D synthesis from UV radiation^(47,51). Other studies have longer intervention periods of 6 months or more, which could not have avoided some cutaneous synthesis. Furthermore, baseline status may be another factor that influences the relative effectiveness factor. The study of Catalano *et al.* had the highest factor of 7.14 in the present review, and the baseline concentration of 25(OH)D of the study participants was higher (>50 nmol/l) than the others⁽⁵⁴⁾. Therefore, the different relative effectiveness seen in different studies may be due to the different characteristics or genotypes of the subjects, or different study designs.

Overall, evidence suggests that dietary 25(OH)D₃ can more effectively increase serum 25(OH)D concentrations than vitamin D₃ and may also be absorbed faster reaching a serum or plasma 25(OH)D plateau earlier than vitamin D₃ supplementation. Furthermore, supplementation with 25(OH)D₃ may also have more benefits to human health compared with vitamin D₃ in a general healthy population. Bischoff-Ferrari *et al.* reported that 20 µg 25(OH)D₃ supplementation over 4 months led to a 5.7 mmHg decrease in systolic blood pressure and improvements in several markers of innate immunity in healthy postmenopausal women⁽⁶²⁾.

For patients with different diseases and receiving long-term medication, studies^(63–65) showed that several drugs (e.g. antiepileptic agents, glucocorticoids, antiretroviral

or anti-oestrogen drugs) interfered with vitamin D metabolism, which resulted in patients being more likely to have low vitamin D status. Thus, it is not only important to increase vitamin D status in the generally healthy population but also in patients with specific illnesses and receiving certain medication. Therefore, the studies using 25(OH)D₃ treatments in patients were also summarised in Table 4^(46,48,53–56), and those studies consistently reported that chronic 25(OH)D₃ supplementation effectively increased serum 25(OH)D concentrations. For example, Ortego-Jurado *et al.* showed a lower daily dose of 8.85 µg 25(OH)D₃ to be more effective than a 20 µg dose of vitamin D₃ for increasing vitamin D status in patients with autoimmune disease who were treated with a low dose of glucocorticoids throughout the year⁽⁵⁵⁾. Similarly, the study of Banon *et al.* showed that a monthly dose of 400 µg 25(OH)D₃ was safe and effective at improving vitamin D status of HIV-infected patients throughout the year⁽⁵³⁾.

Furthermore, supplementation with 25(OH)D₃ may have additional benefits on patients' health. Previously, 25(OH)D₃ was recommended for patients with kidney disease since 25(OH)D₃ has a direct action on bone metabolism⁽⁶⁶⁾. Hahn *et al.* provided a daily 40 µg 25(OH)D₃ and 500 mg calcium supplement to patients who had glucocorticoid-induced osteopenia for 18 months⁽⁴⁶⁾. The treatment markedly increased vitamin D status from 39 to 205 nmol/l. In addition, this study showed that the 25(OH)D₃ treatment improved mineral and bone metabolism. Jean *et al.* also offered haemodialysis patients who suffered from vitamin D deficiency with a daily dose of 16 µg 25(OH)D₃ for 6 months; vitamin D status reached 126 nmol/l from 30 nmol/l, at the same time 25(OH)D₃ supplementation corrected the excess bone turnover⁽⁴⁸⁾. Similarly, a study by Catalano *et al.*⁽⁵⁴⁾ provided 140 µg 25(OH)D₃ supplements for 24 weeks to osteopenic and dyslipidaemic postmenopausal women, and results showed that 25(OH)D₃ improved plasma lipid levels (increased HDL-cholesterol ($P = 0.02$) and decreased LDL-cholesterol ($P = 0.02$)) in osteopenic and dyslipidaemic postmenopausal women when added to an ongoing atorvastatin treatment.

As an alternative to vitamin D-enriched foods, vitamin D fortification of foods may also be an option for tackling vitamin D deficiency throughout the world. In general, fortification of foods refers to mandatory and voluntary fortification. The contribution of vitamin D-fortified foods to vitamin D intake by the public varies considerably between countries as there are different food standard policies⁽¹⁰⁾, and in practice, vitamin D₂ or vitamin D₃ are used for fortification. Evidence from one previous meta-analysis of RCT showed that vitamin D₃ supplementation is more effective at raising vitamin D status than vitamin D₂⁽⁶⁷⁾. However, a further comprehensive systematic review and meta-analysis of thirty-three RCT⁽⁶⁸⁾ showed that the effect of vitamin D₃ supplement on serum 25(OH)D₃ response was limited by the supplemental dose, duration, age of subjects and baseline level. In addition, the meta-analysis showed a greater serum or plasma 25(OH)D increase when the intervention study used a dose of 20 µg/d vitamin D₃ or

even higher, with subjects aged >80 years and an administration period of at least 6–12 months or subjects had lower baseline 25(OH)D status (<50 nmol/l) than subjects aged <80 years, administration period <6 months or subjects had higher baseline 25(OH)D status (≥50 nmol/l)⁽⁶⁸⁾. Therefore, better strategies are needed to raise vitamin D status of the public throughout life, and 25(OH)D₃-fortified foods warrant further research.

Conclusions

Vitamin D insufficiency has become a world problem, especially where sunlight exposure is limited by geographic reasons (latitude), personal characteristics (skin pigmentation, ageing) or behaviour (sunscreen use, cultural reasons). However, there are a few natural foods rich in vitamin D. Thus, vitamin D-enriched foods produced through a food chain approach such as feeding animals vitamin D supplements or vitamin D-fortified foods are needed to guarantee an adequate dietary intake of vitamin D by the general population.

The present review summarised the available and limited number of RCT investigating the effect of 25(OH)D₃ supplementation on serum or plasma 25(OH)D concentration. We concluded that it is difficult to get consensus on the effectiveness of 25(OH)D₃ supplementation relative to vitamin D₃ for raising vitamin D status, due to various influencing factors such as different person characteristics (age, BMI), baseline vitamin D status and time of the year. However, it is unquestionable that 25(OH)D₃ supplementation is more efficient at raising serum 25(OH)D concentrations and also appears to be absorbed faster by than the same dose of vitamin D₃. Second, by reviewing available evidence on vitamin D-enriched eggs, fish or milk, it is practical and possible to increase the vitamin D content of eggs, fish or milk by addition of vitamin D supplements to the diet of poultry, fish or dairy cows. However, the limitations of adding vitamin D to animal feed should be considered in future enrichment studies. Furthermore, there are a few RCT investigating the impact of these vitamin D-enriched foods on improving vitamin D status. Therefore, 25(OH)D₃-enriched or fortified foods should be further explored in the future, and additional RCT should be conducted to investigate the effect of 25(OH)D₃-enriched or fortified foods on vitamin D status of the general population and patients with long-term health conditions.

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Conflicts of Interest

None.

Authorship

J. G. conceived and wrote the manuscript. All authors critically reviewed and approved the final version of the manuscript.

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